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[Continued on next page]

(54) Title: METHOD FOR IN VIVO DETERMINATION ON TESTED ANIMALS OF THE EFFICIENT CONCENTRATION OF DEUTERIUM DEPLETED WATER FOR CANCER THERAPY

(57) Abstract: As per invention embodiment, the method provides Deuterium Depleted Water administering to tested animals before and after tumoral grafting with animal grafts and it takes the following steps: A) Deuterium Depleted Water administering to Wistar outbred rats by diet, with concentrations of 25 ppm D2, 60 ppm D2, and 100 ppm D2, over a period of 60 days, simultaneously to dieting a control group of animals with water having 150 ppm content of Deuterium (tap water), over the same period of time. B) Viability determination for the tumor cells to be grafted, using tripan blue C) Grafting of the animals in the experimental group and the control animals in the 60th day, subcutaneous, with 1 x 107 malign tumor cells in 0.5 cc of normal saline solution of 256 Walker sarcoma (the solid tumor), and T8 Guérinlymphotropic epitelioma (the solid tumor) both of them having cells with a viability over 98%. D) Continuously and long-term administering, by diet, of Deuterium Depleted Water, with concentrations of 25 ppm, 60 ppm, 100 ppm Deuterium, period over which the followings are to be done: a. Starting with the 4th post-graft day the tumor nodules measurement and examination is performed on each 2-3 days; b. Monitoring of animals' physiological condition by weekly weighing, monitoring their food and water consumption, notifying the toxic condition occurrence c. After 60 days, when all the animals in control group are dead, preferable between the 160th and 200th day after graft, the effect produced by administering of established concentrations of Deuterium Depleted Water is observed on the surviving animals homeostasis from experimental groups, respectively the way how humoral immune system and cellular immune system of these animals has been influenced, by performing of a series of examination on immunological condition of the animals, namely: leucocytes formula test to establish lymphocytes and blastic cells levels; hematopoletic marrow tests to establish the plasmocytes and NK-K cells levels. Also, was established the level of the competent immunologic cells in the lymph nods of the tumoral graft zona. E) Determination of efficient concentration of Deuterium Depleted Water for tested surviving animals depending on new homeostasis occurrence, and on the results obtained related to tumoral regression, as well as on cancer curing cases. This method determines the 60 ppm concentration of Deuterium Depleted Water to be the most efficient for cancer therapy and prophylaxis by continuously and long-term administering of this type of water in daily diet.

MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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ategory °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	SOMLYAI G ET AL: "THE BIOLOGICAL EFFECTS OF DEUTERIUM-DEPLETED WATER, A POSSIBLE NEW TOOL IN CANCER THERAPY" DEUTSCHE ZEITSCHRIFT FUER ONKOLOGIE, HEIDELBERG, DE, vol. 30, no. 4, 1998, pages 91-94, XP009006973 ISSN: 0931-0037 cited in the application page 92, left-hand column, paragraph 5 - page 93, left-hand column, paragraph 4	1,2
(WO 95/18545 A (HYD KUTATO-FEJLESZTO KTF; SOMLYAI, GABOR) 13 July 1995 (1995-07-13) cited in the application page 3, line 30 - page 4, line 18	1,2

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International Application No /R02004/000012

gory °	tion) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
J,		resevant to claim No.
-	US 5 855 921 A (SOMLYAI ET AL) 5 January 1999 (1999-01-05) column 4, line 54 - column 6, line 63	1,2
	•	
	•	
		·

INTERNATIONAL SEARCH REPORT information on patent family members

International Application No R02004/000012

		5 1			0047 000012
Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 9518545	Α	13-07-1995	WO	9518545 A1	13-07-1995
			ΑT	205055 T	15-09-2001
		:	AU	691483 B2	21-05-1998
			ΑU	5977794 A	01-08-1995
		!	DE	69428213 D1	11-10-2001
			DE	69428213 T2	13-06-2002
			DK	738114 T3	26-11-2001
			EP	0738114 A1	23-10-1996
			FI	962747 A	02-08-1996
			JP	9511129 T	11-11-1997
			RU	2125817 C1	10-02-1999
			SK	87996 A3	05-02-1997
US 5855921	Α.	05-01-1999	HU	208084 B	30-08-1993
			AT	402692 B	25-07-1997
			ΑT	902992 A	15-12-1996
			BE	1006186 A3	07-06-1994
•			·CA	2122612 A1	13-05-1993
			CH	685282 A5	31-05-1995
			CN	1071836 A ,C	12-05-1993
		•	CZ	9401020 A3	15-12-1994
			DE	4232465 A1	06-05-1993
		:	DK	170832 B1	05-02-1996
			ES	2077530 A1	16-11-1995
		į	FI	941979 A	29-06-1994
		•	FR	2683148 A1	07-05-1993
		* :	GB	2276086 A ,B	21-09-1994
		:	MO	9308794 A1	13-05-1993
		:	IT	1255390 B	31-10-1995
		:	JP	8501275 T	13-02-1996
			JP	3569916 B2	29-09-2004
		÷	KR	253874 B1	15-04-2000
		•	LU	88175 A1	15-03-1993
		•	NL	9220026 A ,B,	01-09-1994
•			NO	941590 A	23-06-1994
		•	SE	513624 C2	09-10-2000
		-	SE	9401406 A	07-06-1994